Establishment of reference intervals of biochemical analytes for South African adults: a study conducted as part of the IFCC global multicentre study on reference values

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Objective: This study was conducted as a part of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) global study for establishing reference intervals (RIs) of common laboratory tests for the South African population considering gender, ethnicity, age and body mass index (BMI).

Methods: The researchers recruited 1 143 apparently healthy volunteers aged 18–65: 551 African (Afr) and 592 non-African (NAfr) (comprising 383 Caucasian and 209 Mixed Ancestry). Serum samples were measured for 40 chemistry and immunochemistry analytes. The standard deviation ratio (SDR) guided the need for partitioning reference values according to gender, ethnicity and age using a threshold of ≥ 0.4 . The latent abnormal values exclusion (LAVE) method was applied to reduce influences of latent diseases before deriving RIs using both parametric and non-parametric methods.

Results: Based on SDR_{sex} , males showed higher albumin, uric acid, creatinine, AST, CK and ferritin. Based on SDR_{RC} , Afr compared to NAfr showed (i) higher total protein, amylase, CRP, immunoglobulin G and A and (ii) lower total bilirubin, total cholesterol, low-density lipoprotein cholesterol (LDL-C), ALT and cholinesterase. Both age-related changes in glucose and LDL-C, and BMI-related changes in ALT, ALP and LDH were more prominent in NAfr. RIs were determined according to gender, age and ethnicity. The LAVE method was effective in lowering the upper RI limits (UL) of nutritional markers such as γ GT and CRP. Compared to the non-parametric method, the parametric method gave narrower confidence intervals of ULs for analytes with skewed distributions.

Conclusion: Establishing RIs by considering ethnicity was essential in many analytes in South Africa. Age and BMI-related changes differed greatly between Afr and NAfr.

Keywords: ethnic variability, age-related changes, BMI-related changes, standard deviation ratio (SDR), latent abnormal values exclusion (LAVE), parametric method, non-parametric method

Introduction

Reference intervals (RIs) play an important role in medical decision-making, especially in diagnosis and monitoring disease.¹ This is why determining reliable RIs is one of the most important missions of any clinical laboratory. A laboratory should derive population specific RIs from healthy individuals from the same population as the patients whose patient samples are analysed by that particular laboratory. Moreover, laboratories are required to regularly verify the RIs in use.¹ However, both the selection and the recruitment of a sufficient number of participants are notably challenging, time-consuming and expensive. In some instances, laboratories use RIs supplied by in vitro diagnostic manufacturers as their patient RIs. However,

these RIs may not always be suitable to the laboratory's patient population.² Differences may be seen in the RIs if the populations live under different environmental and dietary conditions.³ There has been no study to date for establishing RIs for the South African population with consideration to ethnicity, age, gender and body mass index (BMI).

For this reason, we joined the international multicentre project led by the Committee on Reference Intervals and Decision Limits (C-RIDL) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to establish RIs in South Africa. We compared these values to other countries collaborating in the global study (including the United States of America, Turkey, Japan, the United Kingdom, China, India, Saudi Arabia, Argentina, Russia, Philippines, Nepal, Pakistan, Kenya, Nigeria

and Bangladesh). We employed up-to-date analytical methods as well as the latest automated instruments to measure commonly tested analytes. We expected the study to provide us with a unique opportunity to compare South Africa's RIs with those of other countries with widely different demographic profiles. The study also provided us with an opportunity to investigate the controversies over (i) the need for secondary exclusion of individuals with underlying undiagnosed disease conditions by using the latent abnormal values exclusion (LAVE) method and (ii) the use of parametric (P) and non-parametric (NP) methods for the derivation of RIs.^{4,5} The main objective of this study was to derive country-specific RIs for the adult South African population using the common protocol proposed by the C-RIDL.¹

Materials and methods

Study design and recruitment

We obtained ethics approval from the Research and Ethics Committee of the Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology (reference number: CPUT/NHREC: REC-230408-014) as well as the Health and Research Ethics Committee of Stellenbosch University (protocol number: S12/05/147). Each participant received a study information leaflet to inform them of the purpose of the study as well as any risks involved by participating in such a study. Written informed consent was then obtained from each participant.

The study design was adopted from a previously described protocol as used in other countries and it is briefly described below.¹ All participants had to be South African-born citizens regardless of their ethnic origin. Those originally born in the former South West Africa (a province of South Africa in the early 1980s), now Namibia, who continued to live in South Africa were also allowed to participate. The participants had to subjectively feel well and therefore regard themselves as healthy. The target age range was between 18 and 65 years. Participants over the age of 65 were also recruited, however, and although their test results were not used for the derivation of RIs, they were used for analyses of sources of variation (SVs) of RIs. Ideally, participants should not have been taking any medication for chronic conditions. However, for this study we allowed participants who took up to three types of medication for chronic conditions, provided that they did not suffer from any major illness described below. Participants were excluded if they suffered from or had any of the following conditions: (i) diabetes under drug treatment; (ii) reported history of chronic liver or kidney disease; (iii) the presences of a severe disease as indicated by the results obtained in this study; (iv) had been hospitalised or seriously ill during four weeks preceding participation; (v) donated blood in the last three months; (vi) a known carrier state of the hepatitis B virus (HBV), hepatitis C virus (HCV) or HIV-positive; (vii) pregnant or within one year after giving birth and (viii) participated in another research study involving an investigational product during the past 12 weeks. The study was conducted between October 2012 and February 2015 as a multicentre study with a centralised scheme of analysis.¹ Recruitment took place in three core areas located within different provinces in South Africa,

namely: Cape Town, Western Cape; Walter Sisulu University in Mthatha, Eastern Cape; and University of the Witwatersrand in Johannesburg, Gauteng. Each core area was responsible for sample separation, aliquoting and freezing the samples at -80 °C, as described below.

Anthropometric measurements (i.e. weight [kg], height [m], waist circumference [Waist-C; cm] and blood pressure) were taken prior to sample collection. Each participant had to have been seated calmly for at least 20 minutes prior to sample collection to ensure the equal distribution of circulation proteins. BMI was calculated as weight/height² (kg/m²). A comprehensive health status survey was conducted via a detailed questionnaire that was adapted from the one used in the previous Asian project in order to obtain information on BMI, ABO blood type, alcohol consumption, smoking habits, exercise, recent episodes of infection or allergy, and menstrual status.⁶ This questionnaire was completed by each participant while seated as described above. For this study, we added additional items to the questionnaire regarding the frequency of intake of oily fish per week, as well as the duration of sun exposure per day and which parts of their bodies had been exposed to the sun as some Vitamin D (VitD) tests and skin pigment reading measurements were done on a small group of participants to determine their VitD status. Male participants were asked an additional question on dysuria during the past three months while female participants were asked additional questions on the regularity and duration of their menstrual cycle and on hormone replacement therapy.

Sampling and measurements

Participants were requested to avoid excessive physical exertion or exercise three days prior to sample collection. They were also requested to avoid excessive eating and drinking the night before sampling, and to fast for at least 10–12 hours. Approximately 40.0 mL of blood was drawn from each participant into three 9.0 mL SST III Vacutainers® with clot activator and gel, one 4.0 mL lithium heparin tube, one 5.0 mL fluoride tube for glucose analysis and one 4.0 mL EDTA sample for a full blood count. Samples requiring centrifugation were centrifuged at 4 000 rpm for 15 minutes. The samples were then aliquoted and labelled for freezing at -80 °C until the day of analysis. The remote sites at Mthatha and Johannesburg sent their aliquoted samples on dry ice to PathCare Laboratories (ISO 15189 accredited laboratory) in Cape Town for storage at -80 °C until the day of analysis. These samples were analysed together with the samples prepared at the Cape Town site. We chose a centralised scheme of analysis because the samples were analysed collectively in batches of 100–300 samples at a time. Measurements comprised standardised analytes (enzymes, major serum proteins, lipids and electrolytes) as well as non-standardised analytes (ferritin, thyroid function tests, VitD, prostate-specific antigen [PSA]). The analytes that depended on the assay principle of chemical reaction or immune-turbidimetry, were measured using an automated Beckman Coulter DXC analyser for the samples obtained between 2012 and 2013 and using an AU analyser for the samples obtained in 2014. The analytes that depended on

labelled immunoassays were measured by a Beckman Coulter Dxl analyser from the start of analysis in 2012.

For the purpose of this study, dedicated quality control (QC) monitoring was undertaken for both standardised and non-standardised reagents. This was achieved by using multiple samples that were prepared as suggested by the C-RIDL's common protocol and standard operating procedure (SOP).¹ Accordingly, a mini panel of sera from five healthy individuals (two males and three females of different ethnic groups and ages) was prepared and measured with every batch of 100 participant samples over the period of collective measurements. This was done to closely monitor between-day variations of test results.⁵ Furthermore, two panels of sera manufactured by the C-RIDL in 2011 and 2014, respectively, were obtained and analysed (i) to ensure standardisation of RIs through recalibration based on assigned values set to the serum panels, (ii) to align RIs of samples obtained between the two periods and (iii) to allow for the comparison of RIs with those from other countries. The analytes with assigned values in the panels are reported on in the previous reports.⁵

Statistical analyses

All questionnaire data were captured in Microsoft Access and exported to a Microsoft Excel spreadsheet. Patient test results were also exported to a separate Microsoft Excel spreadsheet and these two spreadsheets were then combined and analysed using the same statistical methods as described in the previous studies.⁴ The figures that present the results were all created using StatFlex Version 6 (Artech Co., Ltd, Osaka, Japan) with flexible graphical capability.

Variations of reference values (RVs) by gender (sex), ethnicity (rc), age and BMI were computed through a series of analysis of variance (ANOVAs) as SDRs⁴ (SDR_{sex} , SDR_{rc} , SDR_{age} and SDR_{BMI} , respectively). In the analyses, ethnicity was further divided into two groups: African (Afr) and non-African (NAfr = Mixed Ancestry + Caucasian) because SDR_{rc} values computed in three groups were almost the same regardless of the analytes. Age and BMI were partitioned at 30, 40 and 50 years, and at 22, 26 and 30 kg/m², respectively. SDR_{sex} and SDR_{rc} without gender distinction (M + F) was calculated by a 3-level nested ANOVA, using factors of gender, ethnicity and age. SDR_{rc} for each gender was computed by a 2-level nested ANOVA (ethnicity and age). SDR_{age} without considering ethnicity (All = Afr + NAfr) was computed by a 2-level nested ANOVA (ethnicity and age), and SDR_{age} for each gender and ethnic group was computed by a one-way ANOVA (age). SDR_{BMI} was computed for each gender group and ethnic group in a similar manner than SDR_{age} . SDR values ≥ 0.40 were regarded as a guide for judging the need for partitioning RIs by a given factor.^{4,5}

In this study, it was inevitable that participants with latent diseases of common occurrence such as metabolic syndrome, inflammation and anaemia were included in the sample population. Furthermore, some participants failed to adhere to the basal conditions required for this study or were not truthful in answering the health screening questionnaire as described

earlier. Therefore, to overcome these inevitable problems, the LAVE method was required for some analytes.^{4,7} LAVE is an iterative optimisation method for refining RIs by excluding those subjects who have abnormal results in a set of reference analytes (other than the ones under derivation of the RIs). We set 11 analytes as the reference test items, namely: albumin (Alb), uric acid (UA), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatinine kinase (CK) and c-reactive protein (CRP). We allowed up to one abnormal value within these analytes.

The scheme for derivation of RIs and the 90% confidence interval (CI) of the upper and lower RI limits for this study were essentially the same as reported by Ichihara et al. in 2017.⁷ RIs were determined using both the parametric (P) and the non-parametric (NP) methods. For the P method, the RIs were transformed to a Gaussian distribution using the modified Box-Cox power transformation equation. For the NP method, the RIs were first sorted and then the 2.5 and 97.5 percentile points were determined. The 90% CIs for P and NP methods were predicted using the bootstrap method with a repeated resampling of 100 times. Therefore, the final low limit (LL), upper limit (UL) and midpoint of the RIs were smoothed by taking the averages of their resampled values.

Results

Demography of the participants

The total number of participants recruited was 1 433. However, there was an imbalance in age distribution with a higher proportion in the 18–29 age category for both genders. Therefore, for data analyses, we first applied a random filter to delete 188 participants in order to even out the sample size for each decade of age and gender. In another preliminary step of data validation, we scanned for obvious extreme values analyte by analyte and found one participant with TG > 5.0 mmol/L, one participant with ALT and AST > 130 U/L, two participants with free thyroxine (FT4) > 18 pmol/L, one participant with Alb < 25 g/L, 28 participants with immunoglobulin G (IgG) > 30 g/L and one participant with CRP > 60 mg/L. Therefore, we also deleted these 33 participants with multiple extreme values in one or more analytes. Since we assumed that the high IgG and low Alb are highly suggestive of latent infection, we applied another step to exclude participants with IgG > 20 g/L and Alb < 30 g/L or CRP > 5.0 mg/L, which led to a further reduction in data size of 69. Therefore, the final data size for use in the subsequent data analyses was 1 143 (= 1 433 – 188 – 33 – 69).

Table I summarises the population characteristics of the participants stratified according to ethnicity, gender, age, BMI, Waist-C, systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg) and smoking habit (%). Out of 1 143 participants, 43% were males. The ethnic breakdown of the participants was as follows: 551 (48%) Afr (294 males), 209 (18%) MA (74 males) and 383 (34%) Cau (125 males). Participants with

a smoking habit were 10.3% overall with MA showing the highest percentage (males 16.9% and females 19.7%).

Sources of variations of RVs

The magnitude of three major sources of variation: gender, ethnicity and age were calculated for each analyte as SDR_{sex} , SDR_{rc} and SDR_{age} as shown in Table II. In the analyses, RIs with skewed distributions were made approximately Gaussian using the Box-Cox power transformation equation, then reverse transformed to obtain SDRs in the original scale. The power applied is listed in the second column.

Ethnic-related changes with $SDR_{rc} \geq 0.40$ were noted prominently for TP, TBil, TC, AMY, IgG and IgM in both genders; for urea, ALT and ChE in males; and for AST and CK in females. From Figure 1 and Figure 2, it is noted that the levels of urea (M), TC and LDL-C, ALT (M), AST (F), AMY and ChE (M) were lower in Afr than NAfr. Conversely, the levels of TP, CK (F), IgA and IgM were higher in Afr.

From Table II, we observe a variety of age-related changes that are obviously dependent upon gender and/or ethnicity. $SDR_{age} \geq 0.4$ was found in males for Glu (NAfr) and TC (NAfr), TG (All), and LDL-C (NAfr), while in females for urea (NAfr), UA (Afr), IP (NAfr), TC (All), TG (Afr), GGT (Afr), ChE (Afr), Ferr (All) and IgA (Afr). Based on Figure 1, the direction of changes in values by age was to the side of increase with age in all analytes except for IP which showed a biphasic pattern: decrease until 49 years and then increase afterwards.

From Table II, BMI-related changes are observed in many analytes, which were again dependent on gender and/or ethnicity. $SDR_{BMI} \geq 0.40$ were noted in males for Glu (Afr), TG (Afr), ALT (NAfr), ChE (All) and CRP (Afr), while in females for Glu (All), ALP (NAfr), LDH (NAfr), ChE (NAfr) and CRP (All). From Figure 2, the direction of BMI-related change was, in all analytes, to the side of increase proportionate to BMI. Exceptionally, the opposite direction of BMI-related changes was observed in females for HDL-C (All) and AMY (NAfr), although SDR_{BMI} was less than 0.40. A minor degree of BMI-related changes was also observed in males for AST (NAfr) and GGT (NAfr) and in females for ALT (NAfr) and GGT (NAfr).

Determination of RIs

Based on these findings regarding the influence of gender, age and ethnicity on RVs, we partitioned RVs in multiple ways in deriving the RIs. For age-related change, we chose to partition RVs at 45 years of age because of insufficient sample sizes. For the influence of BMI on RVs, we did not exclude individuals with high BMI. However, for TG, ALT, AST, GGT and CRP, the influence of BMI was reduced by applying the LAVE method as described below. For the analytes with ethnic variability, we first determined RIs without partition by ethnicity for all analytes, and then we determined the RIs of analytes with high SDR_{rc} separately for Afr and NAfr.

As for the calculation methods, we determined the RIs of each analyte using both P and NP methods with/without LAVE. In Figure 3, RIs calculated in four ways were compared for seven representative analytes that were found appropriate to apply the LAVE method because of obvious lowering of upper limits (UL) of their RIs. The comparison of P and NP methods revealed that the 90% CI for UL was generally wider with the NP method. This phenomenon was not only true for the seven analytes shown in the figure, but also for other analytes. Therefore, we only adopted the RIs calculated using the P method in this study. Conversely, the effect of the LAVE method in adjusting the lower limit (LL) or UL was not apparent in other analytes.

Table III shows a list of the RIs determined without partition by ethnicity after consideration of gender and age and the need for the LAVE method. The RIs were partitioned by gender, guided by SDR_{sex} for Alb, UA, Cre, HDL-C, ALT, AST and Ferr. Whereas, for urea, ALP, GGT and ChE, analytes were also partitioned by gender despite low SDR_{sex} because notable age-related changes were observed only in females. However, RIs partitioned by age was derived for TC, TG and LDL-C guided by high SDR_{age} in females.

Table I: Demographic characteristics of participants.

Ethnicity	Gender	Age (years)						BMI (kg/m ²)		Waist - C (cm)		Systolic BP		Diastolic BP		Smoke habits (%)
		N	18-29	30-39	40-49	50-59	60 +	N	M ± SD	N	M ± SD	N	M ± SD	N	M ± SD	
African	M	291	77	79	60	57	18	285	26.1 ± 5.8	276	90 ± 14.6	171	130 ± 17.5	171	83 ± 10.5	6.6
	F	260	80	72	45	46	17	250	27.7 ± 5.9	246	89 ± 14.0	229	126 ± 19.5	228	82 ± 11.3	0.9
Mixed Ancestry	M	74	24	17	15	14	4	72	25.7 ± 3.9	71	89 ± 10.7	71	124 ± 14.7	71	84 ± 10.8	16.9
	F	135	47	30	34	17	7	132	25.3 ± 4.6	130	83 ± 12.2	133	120 ± 18.3	133	80 ± 11.2	19.7
Caucasian	M	125	44	28	28	19	6	124	26.0 ± 3.6	123	89 ± 11.3	124	129 ± 16.7	124	81 ± 11.6	10.8
	F	258	90	49	46	46	27	256	24.3 ± 4.1	254	82 ± 11.5	254	115 ± 14.0	253	77 ± 10.1	9.0
Total		1 143	362	275	228	199	79	1 119	25.9 ± 5.2	1 100	87 ± 13.3	982	123 ± 17.9	980	81 ± 11.1	10.9

BMI – body mass index, Waist- C – waist circumference, BP – blood pressure

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Table II: Effect sizes of variations by gender (SDR_{sex}), ethnicity (SDR_{rc}), age (SDR_{age}) and BMI (SDR_{BMI})

Analyte	Power	(1)	(2)	(3)		(4)	
		SDR _{sex}	SDR _{rc}	SDR _{age} for M	SDR _{age} for F	SDR _{BMI} for M	SDR _{BMI} for F
			M + F (M, F)	All (Afr, NAfr)	All (Afr, NAfr)	All (Afr, NAfr)	All (Afr, NAfr)
TP	1	0.00	0.58 (0.56, 0.60)	0.00 (0.07, 0.00)	0.00 (0.00, 0.21)	0.10 (0.13, 0.00)	0.09 (0.07, 0.12)
Alb	1	0.41	0.20 (0.26, 0.15)	0.23 (0.26, 0.22)	0.00 (0.09, 0.00)	0.20 (0.24, 0.00)	0.15 (0.20, 0.07)
Urea	0.5	0.00	0.46 (0.62, 0.28)	0.00 (0.28, 0.00)	<u>0.30</u> (0.26, 0.43)	0.13 (0.12, 0.15)	0.09 (0.15, 0.00)
UA	0.5	0.81	0.05 (0.21, 0.00)	0.00 (0.27, 0.00)	<u>0.33</u> (0.51 , 0.18)	0.23 (0.26, 0.18)	<u>0.35</u> (<u>0.33</u> , <u>0.38</u>)
Cre	0.5	1.02	<u>0.26</u> (<u>0.34</u> , 0.14)	0.00 (0.04, 0.00)	0.00 (0.00, 0.06)	0.10 (0.00, 0.25)	0.11 (0.08, 0.12)
TBil	0.3	0.00	0.57 (0.69, 0.47)	0.00 (0.28, 0.23)	0.00 (0.00, 0.09)	0.19 (0.21, 0.16)	0.12 (0.13, 0.12)
Na	1	0.00	0.00 (0.00, 0.00)	<u>0.11</u> (<u>0.36</u> , 0.10)	<u>0.20</u> (0.16, <u>0.32</u>)	0.17 (0.16, 0.21)	0.18 (0.19, 0.15)
K	1	0.00	0.23 (0.21, 0.24)	0.00 (0.00, 0.05)	0.00 (0.05, 0.00)	0.13 (0.17, 0.00)	0.14 (0.13, 0.14)
Cl	1	0.00	0.17 (0.21, 0.12)	0.08 (<u>0.34</u> , 0.25)	0.00 (0.09, 0.22)	0.17 (0.18, 0.15)	0.15 (0.16, 0.10)
Ca	1	0.27	0.00 (0.00, 0.00)	0.21 (0.19, 0.25)	0.24 (0.19, 0.27)	0.14 (0.18, 0.00)	0.09 (0.09, 0.07)
IP	1	0.09	0.00 (0.00, 0.00)	<u>0.36</u> (<u>0.35</u> , <u>0.35</u>)	<u>0.32</u> (0.18, 0.43)	0.27 (0.26, 0.29)	0.17 (0.17, 0.17)
Mg	1	0.00	0.00 (0.08, 0.00)	0.14 (0.17, 0.09)	0.20 (0.10, 0.24)	0.12 (0.11, 0.17)	0.11 (0.13, 0.07)
Glu	0.3	0.20	0.19 (0.29, 0.06)	0.45 (0.28, 0.40)	<u>0.37</u> (0.00, <u>0.35</u>)	<u>0.33</u> (0.49 , 0.28)	0.46 (0.53 , 0.46)
TC	1	0.00	0.58 (0.56, 0.59)	0.00 (<u>0.32</u> , 0.66)	<u>0.23</u> (0.48 , 0.48)	0.24 (0.22, 0.28)	0.12 (0.08, 0.15)
TG	0	0.20	0.00 (0.00, 0.00)	0.49 (0.43 , 0.56)	<u>0.38</u> (0.63 , 0.19)	0.40 (0.41 , <u>0.38</u>)	<u>0.30</u> (0.28, <u>0.33</u>)
HDL-C	0.3	0.35	<u>0.28</u> (0.00, <u>0.36</u>)	0.00 (0.00, 0.14)	0.00 (0.00, 0.00)	0.17 (0.21, 0.00)	0.27 (0.23, <u>0.30</u>)
LDL-C	1	0.00	0.70 (0.76, 0.66)	0.00 (0.12, 0.58)	0.00 (<u>0.36</u> , 0.47)	0.29 (0.27, <u>0.31</u>)	0.24 (0.13, <u>0.30</u>)
LIP	0.3	0.00	0.00 (0.00, 0.00)	0.14 (0.14, 0.00)	0.02 (0.16, 0.00)	0.08 (0.09, 0.06)	0.20 (0.04, 0.27)
ALT	0	0.13	0.49 (0.62 , <u>0.35</u>)	0.00 (0.07, 0.16)	0.09 (0.22, 0.28)	<u>0.37</u> (0.20, 0.60)	<u>0.26</u> (0.00, 0.38)
AST	0	0.41	<u>0.38</u> (0.23, 0.48)	<u>0.18</u> (<u>0.30</u> , 0.00)	<u>0.00</u> (0.12, <u>0.37</u>)	0.18 (0.11, 0.28)	0.15 (0.00, 0.21)
ALP	0.5	0.17	0.23 (0.22, 0.23)	0.08 (0.22, 0.00)	0.28 (0.24, <u>0.36</u>)	0.21 (0.14, <u>0.32</u>)	<u>0.38</u> (0.24, 0.48)
AMY	0.3	0.00	0.75 (0.84, 0.67)	0.00 (0.00, 0.00)	0.00 (0.12, 0.00)	0.13 (0.18, 0.00)	0.19 (0.13, 0.24)
LDH	0.5	0.00	0.14 (0.22, 0.00)	0.00 (0.00, 0.00)	0.07 (0.00, 0.09)	0.20 (0.18, 0.23)	<u>0.29</u> (0.00, 0.41)
CK	0	0.52	0.28 (0.00, 0.41)	0.21 (0.16, <u>0.30</u>)	0.00 (0.00, 0.00)	0.11 (0.00, 0.20)	0.09 (0.00, 0.13)
GGT	0	0.24	<u>0.25</u> (<u>0.33</u> , 0.16)	0.25 (<u>0.32</u> , <u>0.34</u>)	<u>0.21</u> (0.41 , 0.10)	0.23 (0.18, 0.32)	0.22 (0.15, 0.29)
ChE	1	0.00	0.40 (0.56 , 0.08)	0.00 (0.18, 0.00)	0.41 (0.43 , <u>0.35</u>)	0.42 (0.41 , 0.44)	<u>0.38</u> (<u>0.31</u> , 0.43)
CRP	0	0.00	0.25 (0.27, 0.23)	0.16 (0.19, 0.12)	0.00 (0.28, 0.00)	<u>0.34</u> (0.40 , 0.19)	0.44 (0.44 , 0.44)
Fe	0.5	0.00	<u>0.35</u> (<u>0.32</u> , <u>0.37</u>)	0.00 (0.11, 0.00)	0.00 (0.00, 0.00)	0.03 (0.11, 0.00)	<u>0.20</u> (0.00, <u>0.30</u>)
Ferritin	0.2	0.65	0.00 (0.11, 0.00)	<u>0.22</u> (0.30, 0.04)	0.56 (0.62 , 0.51)	0.21 (0.19, 0.25)	0.11 (0.14, 0.06)
Tf	0.4	0.31	0.00 (0.00, 0.00)	0.00 (0.11, 0.00)	0.29 (0.28, 0.29)	0.00 (0.00, 0.00)	0.00 (0.02, 0.00)
IgA	0.4	0.00	0.48 (0.40 , 0.54)	0.04 (0.26, 0.25)	0.00 (0.49 , 0.00)	0.00 (0.00, 0.00)	0.22 (0.19, 0.24)
IgG	0.3	0.00	1.02 (1.05, 1.00)	0.00 (0.00, 0.00)	0.00 (0.12, 0.11)	0.00 (0.07, 0.00)	0.00 (0.04, 0.00)
IgM	0.3	0.36	0.12 (0.26, 0.00)	0.00 (0.00, 0.10)	<u>0.30</u> (0.25, <u>0.31</u>)	0.07 (0.00, 0.20)	0.03 (0.00, 0.11)
FT3	1	0.30	<u>0.26</u> (0.16, <u>0.32</u>)	0.15 (0.13, 0.13)	0.00 (0.14, 0.00)	0.16 (0.15, 0.22)	0.15 (0.00, 0.24)
FT4	1	0.00	0.16 (0.00, 0.22)	0.17 (0.24, 0.11)	0.00 (0.00, 0.08)	0.02 (0.00, 0.10)	0.00 (0.00, 0.00)
TSH	0	0.00	0.00 (0.00, 0.00)	0.11 (0.00, 0.21)	0.05 (0.00, 0.12)	0.12 (0.16, 0.00)	0.13 (0.18, 0.08)
Thy	0	0.00	0.20 (0.18, 0.21)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.17 (0.14, 0.20)	0.17 (0.00, 0.09)
TgAb	0	0.00	0.06 (0.12, 0.00)	0.00 (0.00, 0.00)	0.12 (0.13, 0.10)	0.03 (0.01, 0.04)	0.03 (0.00, 0.03)
PSA	0	---	--- (0.16, ---)	<u>0.33</u> (0.27, <u>0.39</u>)	---	0.00 (0.00, 0.07)	---

SDR ≥ 0.4 with no between-sex or between-race mismatch

SDR ≥ 0.4 with between-sex or between-race mismatch

0.3 ≤ SDR < 0.4 with between-sex or between-race mismatch

Definition of mismatch: ΔSDR > 0.15

Race was subgrouped into African (Afr) and non-African (NAfr); All – Afr + NAfr, M – male, F – female

(1) By 3-level ANOVA (sex, race, age)

(2) By 2-level ANOVA (race, age) for each sex

(3) By 2-level ANOVA (race, age) for each sex (for All), then by 1-way ANOVA (age) for each sex and race

(4) By 2-level ANOVA (race, BMI) for each sex (for All), then by 1-way ANOVA (BMI) for each sex and race

Figure 1: Effect of gender, ethnicity, and age on reference values for 16 representative analytes

After partitioning reference values by gender, ethnicity (Afr vs NAFr), and age (divided at 30; 40; 50 years), box-whisker chart (vertical line – median, box span – central 50%, whisker span – central 90%) was drawn for each subgroup. SDRs for between-gender, between-ethnicity (rc), and between-age were shown on top of each graph. SDR_{rc} was computed for each gender (M: male; F: female), and SDR_{age} was computed for each gender and for each ethnic group (Afr; NAFr)

Figure 1: Effect of gender, ethnicity, and age on reference values for 16 representative analytes (continued)

Figure 2: Effect of gender, ethnicity, and BMI on reference values for 8 representative analytes

After partitioning reference values by gender, ethnicity (Afr vs N Afr), and BMI (divided at 22; 26; 30 km/m²), box-whisker chart (see legends for Fig. 1) was drawn for each subgroup. The SDRs for between-gender, between-ethnicity (rc), and between-BMI was shown on top of each graph. SDR_{rc} was computed for each gender (M: male; F: female), and SDR_{BMI} was computed for each gender and for each ethnic group (Afr; N Afr).

Table III: South African derived RI's all analytes all ethnic groups partitioned by age and gender

						90% CI of LL		RI		90% CI of UL		
Item	Unit	Gender	Method	Age	N	LL-L	LL-H	LL	Mean	UL	UL-L	UL-H
TP	g/L	M + F	LAVE (−)	All	1 034	62	64	63	72	82	81	82
Alb	g/L	M	LAVE (−)	All	449	36	37	37	42	49	48	49
		F		All	587	34	35	34	40	47	46	47
		M		All	449	2.56	2.79	2.66	4.66	7.93	7.63	8.32
Urea	mmol/L	F	LAVE (−)	< 45	402	2.01	2.29	2.16	3.92	6.29	6.01	6.57
		F		≥ 45	196	2.39	2.78	2.55	4.72	8.15	7.7	8.74
		M		All	448	196	226	208	344	489	474	508
UA	mmol/L	F	LAVE (−)	< 45	405	145	167	156	249	373	357	391
		F		≥ 45	195	169	195	176	279	466	441	497
		M		All	448	53	58	55	82	110	107	113
Cre	μmol/L	F	LAVE (−)	All	585	44	47	45	63	86	84	88
		M + F		All	1 017	3.1	4.1	3.8	9	22.2	20.7	24.5
TBil	μmol/L	M + F	LAVE (−)	All	1 017	130	131	131	139	144	143	144
Na	mmol/L	M + F	LAVE (−)	All	1 017	130	131	131	139	144	143	144
K	mmol/L	M + F	LAVE (−)	All	1 036	3.6	3.6	3.6	4.3	5.1	5.1	5.2
Cl	mmol/L	M + F	LAVE (−)	All	1 029	96	98	97	105	112	111	112
Ca	mmol/L	M + F	LAVE (−)	All	1 035	2.07	2.12	2.1	2.34	2.58	2.57	2.6
IP	mmol/L	M + F	LAVE (−)	All	1 037	0.75	0.79	0.76	1.07	1.36	1.34	1.39
Mg	mmol/L	M + F	LAVE (−)	All	1 037	0.52	0.67	0.65	0.81	0.94	0.92	0.96
TC	mmol/L	M + F	LAVE (−)	< 45	716	2.7	2.92	2.81	4.51	6.53	6.34	6.84
		M + F		≥ 45	337	3.02	3.34	3.17	5.3	7.66	7.42	7.95
		M + F		< 45	621	0.38	0.43	0.41	0.8	2.42	2.13	2.67
TG	mmol/L	M + F	LAVE (+)	≥ 45	265	0.48	0.57	0.50	1.06	2.90	2.53	3.4
		M		All	446	0.67	0.76	0.71	1.08	1.64	1.56	1.73
HDL-C	mmol/L	F	LAVE (−)	All	583	0.74	0.82	0.78	1.24	1.99	1.93	2.07
		M + F		< 45	719	1.02	1.18	1.11	2.54	4.2	4.04	4.36
LDL-C	mmol/L	M + F	LAVE (−)	≥ 45	337	1.24	1.5	1.4	3.04	5.12	4.88	5.34
		M		All	373	14	15	14	23	42	40	45
AST	IU/L	F	LAVE (+)	All	509	12	13	12	19	31	29	33
		M		All	367	2.9	4.7	3.8	11.8	40.8	33.6	47.4
ALT	IU/L	F	LAVE (+)	All	500	2.7	3.4	2.8	8.2	22.3	20.7	24.5
		M + F		All	1 031	103	113	109	162	229	223	235
LDH	IU/L	M	LAVE (−)	All	443	44	49	47	73	126	119	135
		F		< 45	392	35	40	37	60	110	101	121
ALP	IU/L	F	LAVE (−)	≥ 45	191	37	49	45	74	135	123	155
		M		All	341	14	15	14	32	99	87	111
		F		< 45	253	11	14	13	22	66	56	79
GGT	IU/L	F	LAVE (+)	≥ 45	125	13	14	14	27	90	73	110
		M		All	366	52	65	58	156	569	481	668
CK	IU/L	F	LAVE (+)	All	506	35	44	39	89	271	240	341
		M + F		All	1 017	15	19	17	29	50	47	52
LIP	U/L	M	LAVE (−)	All	450	4.65	5.23	4.88	7.91	11.4	11	11.8
		F		< 45	404	4.39	4.85	4.62	7.09	9.6	9.3	9.9
ChE	kU/L	F	LAVE (−)	≥ 45	196	4.52	5.41	4.86	7.98	11.3	10.9	11.9
		M + F		All	822	0.29	0.34	0.32	1.79	17.76	15.02	21.14
CRP	mg/L	M + F	LAVE (+)	All	822	0.29	0.34	0.32	1.79	17.76	15.02	21.14
Fe	μmol/L	M + F	LAVE (−)	All	1 032	4.7	6.3	5.6	15.2	30.2	29.4	31.6
		M		All	447	9.2	19	13.1	90	374	331	443
Ferritin	μg/L	F	LAVE (+)	< 45	345	2.8	4.5	3.1	23	112	90	134
		F		≥ 45	149	5.3	9.9	7	65	247	209	286
Tf	g/L	M + F	LAVE (−)	All	1 036	1.82	1.9	1.85	2.48	3.53	3.45	3.63

Table III: South African derived RI's all analytes all ethnic groups partitioned by age and gender (continued)

Item	Unit	Gender	Method	Age	N	90% CI of LL		RI			90% CI of UL	
						LL-L	LL-H	LL	Mean	UL	UL-L	UL-H
IgA	g/L	M + F	LAVE (-)	All	1 013	0.9	0.9	0.9	2.2	4.8	4.6	5.1
IgG	g/L	M + F	LAVE (-)	All	1 016	7.3	7.8	7.6	12.5	19.3	19	19.8
IgM	g/L	M	LAVE (-)	All	433	0.35	0.42	0.39	1	2.21	2.06	2.41
		F		All	576	0.45	0.54	0.5	1.28	2.89	2.77	3.08
FT3	pmol/L	M + F	LAVE (-)	All	1 032	3.68	3.85	3.76	4.77	6.03	5.95	6.17
FT4	pmol/L	M + F	LAVE (-)	All	1 035	7.7	8	7.8	10.5	13.9	13.6	14.2
TSH	mIU/L	M + F	LAVE (-)	All	847	0.41	0.5	0.46	1.36	3.63	3.4	3.94
Thyro	IU/mL	M + F	LAVE (-)	All	858	0	0	0	0.13	1.11	0.81	2.23
TgAb	IU/mL	M + F	LAVE (-)	All	960	0	0.04	0.01	0.62	4.7	3.29	336
PSA	µg/L	M	LAVE (-)	< 45	287	0.2	0.24	0.22	0.57	1.71	1.51	1.96
				≥ 45	119	0.14	0.29	0.21	0.93	9.02	5.14	15.06

CI – confidence interval, LL – lower limit, UL – upper limit

Table IV shows a list of the RIs determined for 11 analytes (TP, urea, Cre, TBil, TC, LDL-C, ALT, AMY, ChE, IgG and IgA), which exhibited obvious between-ethnic group differences in RVs. The RIs were calculated separately for Afr and NAfr with consideration to gender, age and the need for LAVE.

Standardisation of RIs

The between-day and within-day coefficient of variations (CVs) of each analyte for both the DXC and AU analysers are shown in Table V. As described earlier, this study measured both panel I and panel II manufactured by the C-RIDL on two separate occasions during the course of this study. In order to align the two set of test results, former results were first converted to the values of

the latter results (AU) using major axis linear regression of the two sets of panel test results.^{5,8} Then, the standardised status of AU assays were evaluated using the major axis regression line between panel test results by AU analyser and standardised values assigned to the panel sera as shown in Figure 4 for 12 representative analytes. The need for recalibration was judged by a ratio of bias for LL or UL (ΔLL or ΔUL) to the SD comprising the RI, which corresponds to between-individual SD according to the following formulae:

$$\Delta LL \text{ ratio} = |LL_- - LL_+| / (UL_+ - LL_+) / 3.92$$

$$\Delta UL \text{ ratio} = |UL_- - UL_+| / (UL_+ - LL_+) / 3.92$$

Figure 3: Comparison of RIs by four calculation methods for seven representative analytes

RIs were calculated by both parametric (P) and non-parametric (NP) methods with/without application of LAVE. Each horizontal bar represents the RI, and the vertical line in the centre corresponds to the mid-point. The shades on both ends of the bar represent 90% CI for the limits of the RI predicted by the bootstrap method.

where $LL_{\pm}$ and $LL_{\pm}$ (or $UL_{\pm}$ and $UL_{\pm}$) represent LL (or UL) with and without recalibration. We set the critical value for ΔLL (or ΔUL) ratio as 0.375 in analogy to the theory of allowable analytical bias in laboratory tests because the numerator of ΔLL (or ΔUL) is a bias or change caused by the recalibration. Based on this scheme, five analytes (Cre, AST, ALT, LDH and GGT) were judged as requiring recalibration of RIs based on the linear regression coefficients.

Discussion

As a part of the global multicentre collaborative project for derivation of RIs, this study was the largest and the first study of this nature for the South African population to establish RIs for the majority of general biochemistry analytes. Moreover, this study was conducted using a standardised and harmonised protocol.¹ In this study, we successfully derived RIs with consideration to ethnic differences in RVs. We proved that the P method is superior in the determination of RIs to the NP method with higher precision of RI limits. Furthermore, we also showed the need for LAVE in derivation of RIs. Previous reports have shown that the P method based on the modified Box-Cox equation remains the method of choice over and above the NP method for the derivation of RIs in almost all settings.⁷

Using the LAVE method has several advantages, namely: (i) truncation of reference distributions does not occur, because this method excludes those participants whose results are outside the RI of any given analyte, other than the analyte for which the RI is being derived, and (ii) there is no effect at all on analytes whose values are on rare occasions just outside the normal RI of the analyte.⁷ There is, however, a disadvantage of using the LAVE method because when applied it reduces the number of participants whose results are included in the final computation of the RIs.

During the process of deriving RIs for this study, we had to adopt a strict policy whereby the abnormal results of participants due to underlying disease had to be excluded. Many pathological conditions exist that can influence each analyte value.² However, we presumed that

Table IV: Selected RIs partitioned by ethnicity, gender and age; Afr vs NAfr

Analyte	Units	Method	Gender	Age	n	AFRICAN						NON-AFRICAN					
						90% CI of LL			90% CI of UL			90% CI of LL			90% CI of UL		
						LL-L	LL-H	LL	UL	UL-L	UL-H	LL-L	LL-H	LL	UL	UL-L	UL-H
TP	g/L	LAVE (-)	M + F	All	502	63	66	64	74	83	85	62	63	62	78	77	79
Urea	mmol/L	LAVE (-)	M	All	262	2.4	2.7	2.5	4.2	6.6	7.6	3.1	3.5	3.3	8.3	7.9	8.9
			F	< 45	165	1.8	2.2	2.0	3.6	5.7	6.6	2.3	2.6	2.5	6.4	6.0	6.8
Cre	μ mol/L	LAVE (-)	M	≥ 45	76	2.3	3.0	2.5	4.2	7.3	9.0	2.3	3.1	2.7	8.1	7.6	8.7
			F	All	263	51	57	54	79	104	114	58	70	65	112	108	116
TBil	μ mol/L	LAVE (-)	M + F	All	238	42	46	44	61	82	89	46	50	48	87	84	91
TC	mmol/L	LAVE (-)	M + F	< 45	499	2.9	4.2	3.5	7.8	17.3	21.3	3.14	4.88	4.3	24.1	22.1	28.4
			M + F	≥ 45	347	2.4	2.7	2.6	4.1	5.9	6.3	3.3	3.6	3.4	6.8	6.6	7.2
LDL-C	mmol/L	LAVE (-)	M + F	< 45	164	2.9	3.2	3.0	4.8	7.1	7.8	3.7	4.2	3.9	7.8	7.5	8.2
			M + F	≥ 45	348	0.8	1.0	0.9	2.2	3.6	3.8	1.6	1.8	1.7	4.5	4.3	4.7
ALT	IU/L	LAVE (+)	M	< 45	164	1.2	1.4	1.3	2.5	4.4	5.1	1.8	2.2	2.0	5.4	5.0	5.7
AMY	IU/L	LAVE (-)	M	All	211	2.7	4.3	3.5	8.8	21.6	32.2	3.8	7.2	5.9	43.7	37.7	53.5
			F	All	207	2.4	2.9	2.6	6.8	15.5	19.8	2.9	4.0	3.6	25.8	23.4	28.5
ChE	kU/L	LAVE (-)	M + F	All	502	39	46	41	84	152	173	25	31	28	111	104	117
IgG	g/L	LAVE (-)	M	All	264	4.5	5.1	4.7	7.4	10.2	11.0	5.5	6.3	5.8	11.9	11.4	12.4
			F	All	240	4.2	4.8	4.5	7.1	9.8	10.4	4.6	5.2	4.9	10.6	10.3	11.0
IgA	g/L	LAVE (-)	M + F	All	483	9.8	10.3	10.1	14.5	19.3	20.1	7.0	7.5	7.3	16.9	16.4	17.6
			M + F	All	483	1.0	1.2	1.1	2.5	4.9	5.7	0.79	0.92	0.84	4.23	3.97	4.50

CI – confidence interval, LL – lower limit, UL – upper limit

Figure 4: Comparison of panel test results with assigned values for standardisation

Major-axis linear regression was used for comparison of our panel test results with values assigned to the serum panel [7]. Need for recalibration of reference values was judged by bias of recalibrated RI as described in the main text.

Table V: List of analytes with methods and between run SD and CV; comparison between the Beckman Coulter DXC, AU and Dxl analysers shown respectively

Analyte	Units	Abbreviation	Method	Beckman Coulter DXC		Beckman Coulter AU	
				Between run SD	Between run CV	Between run SD	Between run CV
Sodium	mmol/L	Na	Ion selective electrode / diluted (indirect)	1.7	0.00	1.2	0.0
Potassium	mmol/L	K	Ion selective electrode / diluted (indirect)	0.3	0.10	0.2	0.1
Chloride	mmol/L	Cl	Ion selective electrode / diluted (indirect)	2.4	0.00	1.6	0.0
Urea	mmol/L	Urea	Urease	1.3	0.30	0.4	0.1
Creatinine	μmol/L	Cre	Modified kinetic Jaffè	11.7	0.20	13.0	0.2
Total Protein	g/L	TP	Buiuret	4.7	0.10	2.8	0.0
Albumin	g/L	Alb	Bromocresol green dye binding	1.8	0.00	1.6	0.0
Total Bilirubin	μmol/L	Tbil	Diazonium salt	2.4	0.30	4.4	0.5
GGT	IU/L	GGT	Gamma-glutamyl-3-carboxy-4-nitroanilide	16.3	0.70	9.0	0.4
ALP	IU/L	ALP	P-nitro-phenylphosphate hydrolysis	19.5	0.30	8.2	0.1
LD	IU/L	LD	Lactate to pyruvate	35.5	0.20	14.4	0.1
Calcium	mmol/L	Ca	Arsenazo III dye	0.1	0.00	0.1	0.0
Magnesium	mmol/L	Mg	Xylidyl blue	0.1	0.10	0.0	0.1
Phosphate	mmol/L	IP	Molybdate hydrolysis	0.1	0.10	0.0	0.0
Glucose	mmol/L	GLU	Hexokinase	0.9	0.20	0.8	0.1
Lipase	U/L	Lip	1, 2-Diglyceride hydrolysis	18.5	0.40	13.2	0.5
Cholesterol	mmol/L	TC	Cholesterol oxidase	1	0.20	1.6	0.3
Triglycerides	mmol/L	Trig	Glycerol phosphate oxidase	0.6	0.40	0.5	0.4
HDL-C	mmol/L	HDL-C	Two phase selective accelerator detergent	0.5	0.40	0.5	0.3
LDL-C	mmol/L	LDL-C	Two phase selective accelerator detergent	0.8	0.30	1.1	0.4
Uric acid	mmol/L	UA	Modified Trinder reaction with uricase	0.1	0.20	0.0	0.1
hsCRP	mg/L	C-RP	Turbidimetry	3.4	0.80	2.0	0.8
Amylase	U/L	AMY	2-chloro-4-nitrophenyl-α-D-maltotrioxide	20.2	0.30	21.4	0.4
IgA	g/L	IgA	Turbidimetry	0.5	0.30	1.3	0.5
IgG	g/L	IgG	Turbidimetry	4.5	0.40	1.5	0.2
IgM	g/L	IgM	Turbidimetry	0.3	0.30	0.5	0.5
ALT	IU/L	ALT	NADH (without P-5'-P)	10.7	0.80	17.4	0.8
AST	IU/L	AST	NADH (without P-5'-P)	4.5	0.20	8.9	0.4
Cholinesterase	kU/L	Che	Butyrylthiocholine hydrolysis	1.2	0.20	1.2	0.2
CK	IU/L	CK	Creatine phosphate dephosphorylation	65	0.50	119.0	0.6
Iron	μmol/L	Fe	2, 4, 6-Tri-(2-pyridyl)-5-triazine chromogen	8.6	0.60	4.1	0.2
Transferrin	g/L	Tf	Turbidimetry	4	1.3	0.4	0.2

				Beckman Coulter Dxl	
				Between run SD	Between run CV
TG-Ab	IU/L	TG-Ab	Two-site immune – enzymatic immunoassay	7.4	4.8
TPO-Ab	IU/L	TPO-Ab	Two-site immune – enzymatic immunoassay	1.1	1.1
TSH	mIU/L	TSH	Two-site immune – enzymatic immunoassay	0.7	0.5
FT4	pmol/L	FT4	Two-site immune – enzymatic immunoassay	1.5	0.1
FT3	pmol/L	FT3	Two-site immune – enzymatic immunoassay	0.6	0.1
Ferritin	μg/L	Fer	Turbidimetry	40.3	0.6
prostate-specific protein	μg/L	PSA	Two-site immune – enzymatic immunoassay	0.6	1.6

if the prevalence is low among apparently healthy individuals, the abnormal results from such participants will not have a major influence on the determination of RIs. Careful consideration was required for those participants with latent but highly prevalent disorders such as metabolic syndrome and undiagnosed diabetes mellitus in the MA population group in South Africa.⁹ As explained earlier, we assumed that high IgG and low Alb is highly suggestive of latent infection, and we, therefore, applied another step to exclude individuals with IgG > 20 g/L and Alb < 30 g/L or CRP > 5.0 mg/L. Furthermore, the LAVE method was applied in our study to exclude those participants with abnormal results attributed to latent disease conditions as the performance of the LAVE method done in previous studies of this nature were well characterised.^{2,10,11}

Ethnic differences in RIs were noted in many analytes. The most conspicuous were ALT, LDL-C, AMY and IgG. For ALT, there have been many reports from epidemiological data based on population sampling schemes whereby very low ALT RIs were observed in African Americans which, in turn, makes them less susceptible to non-alcoholic fatty liver disease.^{12–15} In this study, we confirmed the tendency of low ALT among Afr participants from apparently healthy individuals. We thereby deduct from this finding that a possible genetic factor might contribute to lower ALT in the Afr population, regardless of their location globally. For LDL-C, we found that the RIs of the NAfr group were higher in both males and females in comparison to the Afr population. This tendency has also been shown in other reports.^{16,17}

The differences in RIs between Cau and MA populations were generally smaller when compared to Afr. In this study, we were able to combine the values for Cau and MA to determine generic RIs for these combined population groups, which were named NAfr. Previous research has also demonstrated that the MA population of the Western Cape shares similar RIs to those of Cau descent.¹⁸ The MA ethnic group of Cape Town is a unique population group comprising the original indigenous Khoisan people (32–43%), Bantu-speaking Afr (20–36%), Europeans (21–28%) and Asians (9–11%).¹⁹ According to the last census of 2011, this group makes up 48% of the Western Cape population.²⁰ Although previous studies have reported similar findings in Hispanics who are also of MA,²¹ studies in southern Africa have usually combined those of MA with those of Cau.²² This study, therefore, adds valuable information for this region and could be significant for other MA populations in Africa.

Significantly higher AMY RIs were seen in both males and females in Afr populations when compared to the NAfr group. Perry et al.²³ showed that the copy number of the salivary amylase gene (*AMY1*) is positively correlated with salivary amylase protein level and that individuals from populations with high-starch diets have, on average, more *AMY1* copies than those with traditionally low-starch diets. Rural and urban Afr have been reported to consume large quantities of starchy foods prepared from maize, potatoes, rice and bread.²⁴ One may therefore surmise from these studies that the high AMY RIs in Afr may likely be due to the differences in diet.

This study has also shown that partitioning of RIs by gender was indicated in five analytes, namely: UA, Cre, AST, CK and Ferr. The same findings were seen in the global study in either a prominent or moderate manner.¹⁵ Furthermore, it has been reported that females have more age-related changes in RIs, especially after menopause.¹⁵ Thus menopause-specific RIs have been recommended for many diagnostic biochemical tests when interpreting results for optimal patient management.²⁵

Three Asian studies revealed large regional differences in IgG among healthy individuals.^{7,26,27} The same finding was seen in the harmonised global study¹⁵ as well as in this study. This is probably due to latent infection that could not be elucidated in this study. One of the challenges in this study was to determine which participants to exclude due to the presence of underlying latent disease. By using cut-off values for CRP, Alb and IgG as described earlier, this limitation was overcome with great success.

BMI as a source of variation has shown obvious association with RIs especially in those analytes representing the status of nutrition and inflammation, which was seen in other studies done globally.^{2,8,15} These same changes in RIs due to BMI were seen in our study not only between gender but also between ethnic groups, as described earlier. We see this as an important finding in this study, seeing that the South African population is known to have high obesity rates.²⁸

The major strength of this study was strict adherence to the internationally harmonised protocol elaborated upon by the C-RIDL for recruitment of well-defined healthy individuals, and for use of value-assigned sera panels for standardisation of analytes globally. We made use of up-to-date statistical methods for data analyses. This ensures that the results can be compared to results from other countries participating in the international multicentre project, especially with sub-Saharan countries on the African continent.²⁹ Future research studies should aim to include higher numbers of MA and Cau population groups and strive to generate RIs for adolescents and those younger than 18 years as only a few participants from these two ethnic groups were recruited. This study did not recruit participants younger than 18 and there exists major gaps in the available paediatric laboratory RIs which poses a potential contribution to erroneous diagnosis or misdiagnosis of many diseases in childhood and adolescence.³⁰

Africa has many diverse population groups, and it has been recommended that RIs be generated for each region on the continent. The demographics of South Africa, Africa and the world are constantly changing, and therefore, laboratory professionals should be constantly aware of the influence gender, ethnicity, age and BMI have on the interpretation of laboratory results which has further been substantiated in other research.¹⁸

Conclusion

This study was conducted to establish RIs of major laboratory tests specific to the South African population using the internationally harmonised protocol elaborated on by the IFCC, C-RIDL. SV analyses revealed that ethnic difference in RVs was

very prominent in many analytes including TP, IgA and IgM (higher in Afr), and TBil, TC, LDL-C, AMY and ALT (lower in Afr). Furthermore, there were ethnic differences in the degree of age- and BMI-related changes in many of the analytes. Therefore, in determining RIs it was essential to determine RIs by partitioning RVs based on SDRs for gender, ethnicity and age. For the influence of BMI on RIs of nutritional markers such as ALT, GGT and CRP, the LAVE method was effective to lower their ULs. Using a reference value-assigned sera panel, the RIs were standardised so that they can be used throughout South Africa and can be compared to global RIs as South Africa remains a destination of choice for many foreigners across the globe seeking affordable first world medical treatment.

Data availability

Data from this study are available through a secure online platform upon reasonable request directed to the corresponding author (FCS) and after the approval of a proposal.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

Ethics approval was obtained from the Research and Ethics Committee, Faculty of Health and Wellness Sciences, Cape

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